Arsenic Toxicity Studies in Soil and in Culture Solution[1]

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THE PROBLEM OF arsenic accumulation in soils is one of comparatively recent importance. As agriculture became more intensive, it became necessary to use poisons to combat attacks of certain insects, fungi, and more recently, weeds. Because arsenic is very poisonous to plant enemies and because it is comparatively cheap, it was only natural that it should have found general use. The arsenic so used has for the most part accumulated in the upper soil layers, and sooner or later becomes a menace to crop production. This paper is concerned with this problem particularly with reference to Hawaiian soils.

HISTORICAL REVIEW

Early work.—Toward the end of the last century and the beginning of the present century, some attention was given to the possibility that arsenic might poison the crop itself. In 1894, Lyttkens conducted experiments at the Halmstead Experiment Station in Sweden which showed plainly that the arsenic in the soil is a strong poison to plants.

Stoklasa (1898), however, showed that while arsenic could not replace phosphorus as an essential element, it had stimulative effects on the development of the assimilation organs of the oat plant. The issue thus became confused. While some presented evidence that arsenic was a poison to plants, others presented equally convincing evidence that arsenic stimulated plant growth. Bouilhac (1899) reported that a number of fresh-water algae absorb arsenic acid from arsenates without apparent injury, and the growth of one appeared more favorably influenced by arsenic acid than by phosphoric acid.

The fact that arsenic is a natural component of most soils and not uncommon in plants tended to reduce the force of criticism against the use of it. Zuccari (1914), working in Italy, analyzed 20 soil samples varying in physical and chemical composition and taken from different depths in different geological formations and at varying elevations; these samples showed an arsenic content varying from 0.187 to 6 parts per 100,000 of soil, being largest in soils containing the most iron compounds and varying almost directly with iron content.

Reichert and Trelles (1922) analyzed 20 soils from different parts of Argentina and found all but one to contain arsenic, varying in amounts from 0.1 to 2.25 mg. per 100 gm. of soil.

Williams and Whetstone (1940) analyzed a wide range of soils for arsenic and found the range of naturally occurring arsenic to be between 0.3 and 40 ppm. Vegetation found growing on these soils ranged from less than 0.1 ppm to 10 ppm.

Greaves (1934) analyzed, for total arsenic, water-soluble arsenic, and various soluble salts, 50 orchard soils which had been in cultivation for some time and which varied widely in chemical, physical, and biological properties. Total arsenic varied from 7.2 to 367.2 pounds per acre foot. The water-soluble arsenic varied from 0.7 to 31.9 pounds per acre foot.

Greaves (1913*a*) earlier reported that some virgin soils contain arsenic in appreciable quantities which comes from the decay of native rocks. Many cultivated orchard soils contain it in large quantities, but he found no uniform relationship between the total quantity in different soils and the water-soluble arsenic of these soils. He considered that the solubility of arsenic is governed largely by the salts in the soil and the form in which the arsenic is applied.

Thus, with comparatively high arsenic amounts not uncommon as natural components of soils, opinions on the danger from the use of comparatively small additions continued to be diverse.

Headden (1909) was perhaps the first to recognize the serious dangers which may accrue to orchard trees from continued use of arsenical insecticides. In fact, he set off a heated controversy which has raged ever since. In other reports (1908; 1910), he recognized three types of poison-

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ing, one corrosive, one systemic, and one which was an arsenic-lime poisoning. He pointed out that arsenical deposits in the soil are for the most part insoluble, but warned that the soluble fractions had already passed the danger limits in certain areas. He recognized that absorption of arsenic by the roots takes place. He further pointed out that salts such as $NaCl$, Na_2SO_4, and Na_2CO_3 are all capable of making lead arsenate soluble in the soil solution and that lime salts do not prevent the solution of arsenates.

Headden was joined in his efforts by Swingle and Morris (1911), who reported that serious injury may result to apple trees from the application of insoluble arsenicals and that recent wounds through the outer bark, functional lenticels, and dormant buds permit the absorption of arsenic in solution.

Greaves (1913) observed that water-soluble arsenic may exist in soils to the extent of 82 ppm without entirely stopping ammonification and nitrification. He considered it improbable that lead arsenate, zinc arsenite, or arsenic trisulfide would ever be applied to agricultural soil in quantities sufficient to be injurious to soil bacteria.

Greaves and Anderson (1915) reported actual stimulation of soil flora by soluble arsenic in soil at 10 ppm. Toxicity began at 40 ppm and nitrogen fixation was entirely stopped at 250 ppm. The quantity of 10 ppm which they reported as causing stimulation exceeds that found in most soils, and they concluded that arsenic will stimulate instead of retard bacterial activities of soil. Greaves (1916) reported that arsenic cannot replace phosphorus in the vital process of nitrogen fixing, but it can, in some manner, liberate phosphorus from its insoluble compounds.

Swingle (1920) investigated the effect of arsenic on species of soil bacteria responsible for important chemical changes such as ammonification and nitrogen fixation. Contrary to the work of Greaves, Swingle's results showed that all the arsenical compounds used were germicidal, but in different degrees.

Green and Kestell (1919) found bacteria which are resistant to arsenic to be infrequent in soil and air, but fairly frequent in feces. Of the twelve or more resistant species examined, only two showed any chemical activity toward arsenic: one, which oxidizes arsenite to arsenate, and another, which reduces arsenate to arsenite. No relationship was discovered between arsenate reduction and nitrate reduction.

McGeorge (1915*a*; 1915*b*) appears to have been the first in Hawaii to recognize the possible deleterious effects which may result from the use of sodium arsenite as a herbicide, a practice then coming into general use in the Territory. His researches involved a study of the effect of sodium arsenite on the growth of millet, cowpeas, and buckwheat and on the physical, chemical, and biological activities in heavy red clay, brown clay, and highly organic silt soils. He found that the effect of sodium arsenite on plant growth depends upon the resisting power of the plant and upon the chemical and physical nature of the soil. In small quantities, the compound stimulated plant growth in most instances, but when added at the rate of 0.1 to 0.25 per cent, it made plant growth virtually impossible.

Sodium arsenite, he discovered, materially altered the mechanical condition of the soil, its action being primarily that of a deflocculating agent checking the movement of water.

Sodium arsenite, McGeorge showed, was strongly fixed by the soil, even resisting the washing of heavy rains. An analysis of a sample of soil from a tract of land sprayed three times a year for 5 years, at the rate of 5 pounds of sodium arsenite per acre per application, showed all the arsenic to be present in the top 4 inches of soil. The fixation of arsenites by the soil involved chemical reactions consisting of replacement of solution of iron, calcium, magnesium, and humus, owing in part to a hydrolysis of the sodium arsenite in solution and in part to a combination with the dibasic and tribasic elements, to form the relatively insoluble arsenites and arsenates.

Brenchley (1914*a;* 1914*b*) distinguished between higher and lower forms of plant life in their reactions to arsenic. In certain algae, stimulation may result from the presence of arsenic compounds. Some fungi apparently are able to live in the presence of arsenical compounds. On higher plants, the toxic effect of arsenic was found much more marked with arsenious acid and its compounds than with arsenic acid and its derivatives. Using peas and barley, she could observe no stimulation even with the smallest quantities used.

Morris and Swingle (1927) reported that the addition of small amounts of soluble arsenical compounds to potted plants caused serious injury to most of the plants under test. The authors concluded that the incorporation of arsenical compounds in the soil is a dangerous practice, and may cause considerable injury as the concentration increases. They further concluded that beans and cucumbers were very susceptible, whereas cereals and grasses were more resistant.

To judge from the material so far presented, it appears that the use of arsenic has several problems associated with it. The preponderance of opinion is that arsenic is harmful to higher plants but that when the arsenic occurs in very small concentrations, it may cause some stimulation. Contrary opinion on the latter point is, however, rather substantial. The stimulating effect of arsenic on algae and fungi seems not uncommon, but its favorable influence on soil organisms regarded as useful to agriculture is at least questionable. While the argument was going along on these somewhat academic lines, great quantities of arsenic were applied to orchards and fields. As Headden and Swingle had warned, the continued application and the resulting accumulations of arsenic in the surface layers of soil and its retention there resulted in crop reduction or failure in Washington as reported by Vandecaveye, Horner, and Keaton (1936), in South Carolina as reported by Albert and Paden (1931) and Cooper *et al.* (1931), and in Louisiana as reported by Reed and Sturgis (1936). During the past 15 years, experimental work on arsenic has been directed to several problems: the contamination of plant parts which are used as animal or human food, the levels at which arsenic in soil may cause a reduction in crop yields, and methods which might be used to render a poisonous soil suitable for renewed crop production.

Recent work: Arsenic in food.—Chorley and McChlery (1935), interested in the poisoning of fowls following their consumption of poisoned locusts, reported that when arsenic is administered in small quantities such as occur on sprayed grasshoppers, a domestic fowl can tolerate comparatively large doses over a long period without any visible ill effects.

Franke and Maxon (1936) gave rats intraperitoneal injections of disodium acid arsenite and disodium acid arsenate, as well as other chemicals. The minimum fatal doses were defined as the smallest doses which would kill 75 per cent or more of the animals in less than 2 days. The minimum fatal doses of the arsenite were 4.25–4.75 mg. of arsenic per kilogram of weight, whereas for the arsenate, the fatal dose was 14–18 mg. per kilogram.

Groves, McCulloch, and St. John (1946) concluded from their studies that lead arsenate spray residues are much less toxic to swine than has generally been supposed. One pig consumed the spray residue from 1,007 kilograms (over 1 ton) of heavily sprayed apples which contained 114.8 gm. of lead arsenate in the form of spray residue. The pig gained in weight approximately as much as the control pig, and no abnormalities were apparent in blood studies on it. Data showed that of the edible portions of the pigs which were fed large quantities of lead arsenate, only the livers contained more lead than the 7.14 ppm legal limit, and none of the organs analyzed contained more than the limit of 3.57 ppm of As_2O_3.

The effect which spray residues on plant parts and metabolized arsenic contained within plant tissue may have on higher animals, including humans, has been receiving increasing attention. Talbert and Tayloe (1933), reporting results of feeding spray chemicals to rats, concluded that if it may be assumed that the spray chemicals have an effect upon man similar to that which they have on albino rats, it is their opinion that there is little likelihood that a human being would consume as spray residue on apples, sprayed and handled in the usual manner, enough arsenic either at one time or over an extended period to be injurious.

Coulson, Remington, and Lynch (1934) compared the bodies of rats fed for 3 to 5½ months on diets of varying arsenic content derived (1) from natural shrimp and (2) from added arsenic trioxide. The bodies of animals which had received the largest amount of arsenic, 17.9 mg. per kilogram, in the form of shrimp contained at least four times as much arsenic as the stock diet controls, whereas those which received approximately the same quantity of arsenic in the form of arsenic trioxide contained 55 to 65 times as much as the controls. An even greater difference between the storage of arsenic from the two forms was shown during the first 3 months than after 5½ months, a fact suggesting that the rats receiving the inorganic arsenic had at some period during the first 3 months reached an equilibrium, after which no further storage took place.

There was no retardation of growth in any of the arsenic fed animals nor any observable differences in their physical vigor or appearance, and in none of them was there any histological evidence of injury to the spleen, liver, or kidney due to the feeding of arsenic at the level employed.

These authors (1935) further emphasized the difference between metabolized and inorganic arsenic in foods by showing that arsenic as present in shrimp was found to be far less available for storage in young rats than when fed at the same level as As_2O_3. During the first 3 months of their feeding trial, 18 per cent of the As_2O_3 incorporated in the diet at a level of 17.9 mg. per kg. was stored as against 0.77 per cent for the same amount of arsenic in shrimp. The total amount stored was not significantly increased by feeding the element for an added 9 months. There was no evidence of toxicity from the arsenic in either form after 12 months of feeding.

In two human subjects studied by these authors, the ingestion of shrimp in amounts furnishing 1,180 and 980 gammas of arsenic was followed by rapid and complete elimination of the arsenic. Inorganic arsenic, although excreted more slowly than shrimp arsenic, was apparently eliminated more completely by these subjects than by the rats. These results are considered to be of interest not only for the light which they throw on the metabolism of arsenic, but also as additional evidence that the manner in which inorganic elements are used in the body depends upon the source or form in which these elements are presented.

It appears, therefore, that arsenic which has been metabolized by an organism—that is, arsenic which the organism has absorbed and made a part of itself—is less dangerous to animals than is inorganic arsenic such as may appear on the leaves of plants shortly after spraying. This latter arsenic is quite poisonous to livestock, as most sections of an agricultural country recognize. In an effort to determine how much arsenic plants might absorb and remain normal in appearance, Machlis (1941) grew bush beans and Sudan grass in culture solution. He presented striking evidence that the bean plant may absorb arsenic far in excess of legal tolerance and yet show no reduction in growth.

To discover the factors which may or may not make the use of arsenic-contaminated food safe for animal or human consumption requires further critical studies. On the basis of recent work it appears, however, that there has been more emotion and less knowledge about this subject than is needed for an understanding of it.

Recent work: Arsenic accumulation in soil, its effect on crop production, and corrective measures.—Swingle (1923), in an effort to test the effect of prolonged application of arsenic on plant growth, applied various arsenicals to plots of ground in the spring of the year, and the effect on crop production was noted. After 7 years of such procedure, beans and cucumbers made little growth, while wheat and timothy grew fairly well. No further applications of arsenic were made for 6 years and at the end of that time it was found that very little of the arsenical had been removed by rains or irrigation. Furthermore, it was found very difficult to get the land back into condition for cropping.

Paden and Albert (1930), working in South Carolina, reported a relationship between the unproductivity of certain soil types and the accumulation of soluble arsenic in the soil resulting from heavy applications of calcium arsenate. Lime improved the growth of cotton in the poisoned soil. Soils which were relatively low in iron and other materials which would be expected to render arsenic insoluble were found to be the most seriously affected by the arsenates.

Albert and Arndt (1931) found the concentration of soluble arsenic as measured by collodion bag dialyzates to be a more reliable index of arsenic than is the total arsenic present in the soil. In greenhouse experiments, the addition of 1 ppm of arsenic definitely retarded root and top growth of cowpeas. It was observed that the concentration of 1 ppm of soluble arsenic as measured by the collodion bag test was not unusual in soil which had been receiving doses of arsenates. Liming and the use of fertilizer along with iron and clay compounds of the soil played an important role in rendering arsenates harmless to sensitive crops.

Hurd-Karrer (1936) believed as a result of field tests and culture solutions that phosphate application will reduce or prevent arsenic injury to plants where the soil type is such as to retain the phosphate in available form.

Heggeness (1940), growing tomatoes in culture solution, found no evidence of toxic stimulation, even the most dilute solution (½ ppm) reducing the yield by 20 per cent. Arsenic toxicity in the tomato was partially dependent on phosphate availability.

Keaton (1938) studied the oxidation-reduction potentials of arsenate–arsenite systems in sand and soil mediums. In these soils arsenic was fixed by absorption and combination, but it was observed that a higher percentage of arsenate than arsenite was fixed by these soils. The addition of iron to the system when the original ratio of arsenate to arsenite was unity increased the redox potential independent of the medium used. In the two soils studied the colloidal fraction possessed a greater reducing capacity and a lower potential than the soil from which it was extracted.

Keaton and Kardos (1940) attempted treatment of orchard soil to overcome toxicity of arsenic residues. Their studies indicate a relationship between the oxidation-reduction potentials of the soils treated and the conditions of plant growth. The addition of ferric oxide caused an increase in the redox potential. Alumina produced no effect in oxidation or reduction. Soils with a high colloid content were characterized by low potential and small percentage oxidation. They suggested that poisoned soils be treated with some mild oxidizing agent capable of arsenic fixation, and named iron oxide as such an agent.

Kardos, Vandecaveye, and Benson (1940) reported that severely toxic soils have been rendered productive by making applications of 3 to 4½ tons of *ferrous* sulfate per acre.

EXPERIMENTAL WORK

In order to study certain phases of arsenic toxicity, the experiments reported in this paper were undertaken. Data will be presented and discussed under the following headings:

Part I. The comparative toxicity of trivalent and pentavalent arsenic on bean (*Phaseolus vulgaris* L.), Sudan grass (*Sorghum vulgare* Persoon var. *sudanense* [Piper] Hitch.), and tomato (*Lycopersicon esculentum* Miller).

Part II. The effect of different phosphorus levels on the toxicity of trivalent and pentavalent arsenic on bean, Sudan grass, and tomato.

Part III. The influence of repeated croppings of bean, Sudan grass, and tomato on a red residual soil and on a black alluvial soil treated with various increments of arsenic.

MATERIALS AND METHODS

Culture solutions.—For Part I and Part II mentioned above, water cultures were used. A total of 30 crocks was used for each of the three species—10 crocks at each of three phosphorus levels—with each crock having a known concentration of arsenic in the form of sodium arsenite in the study of the trivalent form and sodium arsenate in the study of the pentavalent form.

The experiments were conducted in a greenhouse of the University of Hawaii Agricultural Experiment Station. Four-gallon size glazed crocks were used for the Sudan grass and tomato samples, and 2-gallon size for the bean. Distilled water was used throughout and continuous aeration maintained. The plants were held in corks set into lids which fitted over the crocks.

The tomato and Sudan grass seeds were germinated on cheesecloth and transferred when large enough to facilitate handling without injury. The bean seeds were germinated in black sand and transplanted as soon as was practicable.

All the plants were given complete nutrients for 2 weeks. The following solutions were used:

	TOMATO	SUDAN GRASS	BEAN
$Ca(NO_3)_2$	.005M	.005M	.005M
KNO_3	.005M	.005M	.005M
$MgSO_4$	.002M	.002M	.002M
$CaCl_2$	.003M	.003M	——
KH_2PO_4	.001M	.001M	.002M

Iron (as $FeSO_4$) and standard amounts of copper, manganese, zinc, and boron were added.

After 2 weeks passed the solutions were changed, but the nutrients as listed above were maintained with the exception of the phosphate, which was added in the amount required in the experiment. The arsenic increments were added as planned, and the plants were selected for uniformity and thinned to three plants per crock for the bean and Sudan grass and two plants for the tomato. All solutions were changed weekly, and the levels of all salts were maintained as required in the experiment.

Plants which did not live until the end of the experiment were removed when they died. All others were allowed to grow for 3 to 4 weeks after the original treatment; then they were harvested. Green weights of the plant tops were taken, after which the plants were dried and dry weights taken. The material was ground in a Wiley mill and stored for subsequent arsenic and phosphate analyses.

Methods of analysis.—The method of Cassil and Wichmann (1939) was used in making the arsenic determinations reported in this paper. For the phosphate determinations, the Truog and Meyer (1929) modification of the Deniges method was used.

The dry plant material was digested as follows: Two grams or less of the sample were added to 125 cc. Erlenmeyer flasks, followed by 10 cc. of H_2SO_4 and 20 cc. of HNO_3. The flasks were allowed to stand for several hours, then heated slowly. Five-cc. increments of HNO_3 were added, and the digestion continued until the solution was clear and yellow. A final 5 cc. of HNO_3 was added with 5 drops of perchloric acid and heat was applied until dense white SO_3 fumes were evolved. The flask was removed and cooled, and the solution made up to 125 cc. From this, suitable aliquots were taken for the arsenic and phosphate determinations.

DATA AND DISCUSSION

Part I. Comparative Toxicity of Trivalent and Pentavalent Arsenic

In Tables 1, 2, and 3 are presented data for experiments using the three species treated with sodium arsenate (Na_2HAsO_4.

TABLE 1. TOMATO

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), AS WELL AS DRY WEIGHTS OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF PENTAVALENT ARSENIC (AS SODIUM ARSENATE) AT DIFFERENT PHOSPHATE LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.0	27	trace	0.72
1	30	5.0	.67
2.5	34	10.6	.61
5	21	22.5	.75
10	18	42.2	.75
15	10	76.5	.72
20	10	81.3	.67
25	6	93.0	.50
30	3	106.3	.72
40	2	162.5	.60
Medium phosphorus level (P = 60 ppm)			
0.0	28	0	.75
6	29	6.7	.72
15	19	17.2	.84
30	18	49.7	.84
60	9	74.1	.69
90	4	120.1	.63
120	2	193.7	.60
High phosphorus level (P = 120 ppm)			
0.0	28	trace	.72
12	23	12.2	.78
30	19	26.6	.69
60	11	61.3	.64
120	6	103.9	.60

$7\ H_2O$), and in Tables 4, 5, and 6 the data for the same species treated with sodium arsenite ($NaAsO_2$).

Since it will be shown later that the phosphorus level has an important influence on arsenic toxicity, comparisons between arsenate and arsenite must be made within series of similar phosphorus concentration. Comparisons of the arsenate and arsenite experiments at the medium phosphorus level (P = 60 ppm) indicate marked differences between the action of pentavalent and of trivalent forms.

The tomato plants growing in 60 ppm of pentavalent arsenic accumulated 74.1 ppm of arsenic in their tops, yet were normal except in size. Tomato plants grown in solutions containing 90 ppm and 120 ppm of pentavalent arsenic lived to the end of the experiment despite accumulations of 120 ppm and 194 ppm of arsenic in their tops. The lethal concentration was between 120 ppm and 150 ppm in the culture solution. In the arsenite series, on the other hand, concentrations of 7 ppm and 11 ppm in the solution very nearly stopped growth, although the plants on analysis showed only 12.5 ppm and 24.1 ppm of arsenic respectively in their tops. Even the low level of 3¼ ppm in the culture caused marked stunting; the lethal concentration was 11 to 15 ppm. Approximately 10 times as much pentavalent arsenic is required in the culture solution and in the plant tissue as trivalent arsenic to produce equivalent injuries to tomato plants.

TABLE 2. SUDAN GRASS

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), AS WELL AS DRY WEIGHTS OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF PENTAVALENT ARSENIC (AS SODIUM ARSENATE) AT DIFFERENT PHOSPHATE LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.0	40	trace	0.61
1	23	13.5	.53
2.5	21	19.1	.56
5	11	47.4	.42
10	5	67.2	.44
15	2	88.8	.42
20	2	103.1	.53
25	2	139.1	.54
Medium phosphorus level (P = 60 ppm)			
0.0	37	trace	.72
6	29	29.4	.75
15	18	41.0	.75
30	8	74.1	.59
60	4	98.4	.56
90	2	513.1	.66
High phosphorus level (P = 120 ppm)			
0.0	36	trace	.84
12	28	12.8	.91
30	20	49.7	.78
60	14	84.4	.59
120	6	142.3	.52

TABLE 3. BEAN

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), AS WELL AS DRY WEIGHTS OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF PENTAVALENT ARSENIC (AS SODIUM ARSENATE) AT DIFFERENT PHOSPHATE LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.00	50	trace	0.64
.1	47	2.5	.55
.25	38	2.8	.51
.5	38	2.5	.50
1.5	28	10.3	.66
2.0	15	13.7	.58
2.5	11	20.0	.53
Medium phosphorus level (P = 60 ppm)			
0.0	44	trace	.59
.6	48	1.6	.59
1.5	43	2.7	.60
3	35	4.1	.63
6	38	5.1	.66
9	26	7.2	.61
High phosphorus level (P = 120 ppm)			
0.0	57	trace	.63
1.2	54	1.9	.59
3	38	2.7	.66
6	37	5.0	.63
12	16	38.0	.72

The Sudan grass plants growing in solutions containing 30 ppm and 60 ppm of pentavalent arsenic were apparently normal except for size, although they accumulated 74.1 and 98.4 ppm of arsenic in their tops. Marked injury was noted at 90 ppm; the lethal concentration was between 90 ppm and 120 ppm. With trivalent arsenic, however, a concentration as low as 3¼ ppm of arsenic in the culture solution resulted in cessation of growth, although the plant tops showed only 14.9 ppm of arsenic. The lethal concentration in the culture solution of the trivalent arsenic was between 11 ppm and 15 ppm. Sudan grass can tolerate approximately 10 times as much pentavalent arsenic in the culture solution and in their tissues as trivalent arsenic.

The bean plants were very intolerant of arsenic in any form. They grew quite normally, however, in solutions containing 9 ppm of pentavalent arsenic, from which they accumulated 7.2 ppm of arsenic in their tops. The lethal concentration was between 12 ppm and 15 ppm of pentavalent arsenic. On the other hand, growth was prevented by a concentration of 2.25 ppm to 2.85 ppm of trivalent arsenic, despite an accumulation of only 4.9 ppm of arsenic in their tops. The lethal concentration of trivalent arsenic in the culture solution was between 2.85 ppm and 3.6 ppm. Trivalent arsenic is roughly four times as toxic to bean plants as is the pentavalent form.

Comparisons made between the arsenate and arsenite series at the high and low phosphorus levels show results similar to those discussed above for the intermediate phosphorus level.

The two forms of arsenic differ not only in lethal concentrations, but also in their immediate action on plant tissues. Trivalent arsenic has a violent action, causing complete disintegration of the roots and burning of the tops in 1 or 2 days in lethal concentrations. Pentavalent arsenic, on the other hand, often takes several days to produce any response other than wilting, even in concentrations that eventually prove lethal.

Part II. The Effect of Different Phosphorus Levels on the Toxicity of Trivalent and Pentavalent Arsenic

It was suggested by Hurd-Karrer (1939) that a relationship exists between arsenic toxicity and phosphorus availability. She used sodium arsenate in culture solutions at different phosphorus levels on studies with the oat plant and came to the conclusion that "in general, the arsenic was definitely toxic in the presence of less than four times as much phosphorus but non-toxic when there was more than four times as much."

Based on that observation, investigations were undertaken to compare the effect of phosphorus level on trivalent and pentavalent arsenic to determine whether the decrease in toxicity was due to decreased absorption of the toxic element in the presence of large amounts of phosphorus or whether it was due to some inhibitory effect which phosphorus might have on arsenic toxicity after absorption by the plant tissues.

Bean, Sudan grass, and tomato plants were grown in culture solutions at three phosphorus levels—10 ppm, 60 ppm, and 120 ppm—and subjected to treatment with sodium arsenate and sodium arsenite.

In Tables 1, 2, and 3 are recorded the data for the tests with *pentavalent* arsenic. It can be seen that an increase in the phosphorus level markedly reduced the amount of pentavalent arsenic absorbed, and resulted in better growth. For example, the tomato plants grown in low-phosphorus solutions containing 30 ppm of arsenic contained 106.3 ppm of arsenic in their tops, as compared to 49.7 ppm and 26.6 ppm in plants grown in medium- and high-phosphorus solutions, respectively, containing similar amounts of arsenic.

Similarly, increases in the phosphorus level reduced the absorption of arsenic by Sudan grass. The analyses of plant tops showed 88.8 ppm, 41.0 ppm, and 20.0 ppm of arsenic from the low-, medium-, and high-phosphorus solutions containing 15 ppm of arsenic.

The relationship of the phosphorus level to arsenic absorption by the bean is not so decisive. The bean has a very small range of tolerance to arsenic and, as a result, the magnitude of the differences between the various cultures is correspondingly small. The beans showed, for example, 10.3 ppm, 2.7 ppm, and 2.0 ppm of arsenic in their tops when grown in low-, medium-, and high-phosphorus solutions, respectively, containing 1.5 ppm of arsenic.

As might be expected, a reduction in the absorption of the toxic element by the plant resulted in better plant growth. The controls showed no significant differences in the dry weights at the three phosphorus levels, indicating that 10 ppm of phosphorus were adequate for the three plants studied. Analysis of the plant tops showed no significant differences among plants grown in low-, medium-, and high-phosphorus levels. Furthermore, the dry weights seemed to correlate with the arsenic concentration in the plant, irrespective of the phosphorus level of the culture solution. These considerations would seem to indicate that the phosphorus in the culture solution is effective in inhibiting the absorption of pentavalent arsenic by the plant but not in reducing the toxicity of the element within the plant.

Hurd-Karrer (1939) suggested that arsenates would be non-toxic if the P : As ratio were more than 4 : 1. Although a high phosphorus level greatly reduced the toxic effects of a given concentration of arsenic in the culture solution by reducing its absorption by the three plants studied here, it did not prevent injury. For example, tomatoes growing in solutions in which the P : As ratio was 10 : 1 (120 ppm : 12 ppm) had a dry weight of 23 grams as compared to 28 grams in the control—a reduction of 18 per cent. Sudan grass in solutions containing 120 ppm of phosphorus and 12 ppm of arsenic (P : As = 10 : 1) weighed 28.5 grams as compared to 36 grams in the control—a reduction of 21 per cent. Bean plants growing in solutions containing 6 ppm of arsenic and 120 ppm of phosphorus (P : As = 20 : 1) weighed 37 grams as compared to 57 grams in the control—a reduction of 35 per cent. A high phosphorus level reduces but does not prevent the absorption of pentavalent arsenic. The degree of injury depends on the amount of the toxic element absorbed.

The data for the studies made with *trivalent* arsenic are recorded in Tables 4, 5, and 6. The results indicate that the action

TABLE 4. TOMATO

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF TRIVALENT ARSENIC (AS SODIUM ARSENITE) AT DIFFERENT PHOSPHORUS LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.00	29	trace	0.66
.25	30	trace	.67
.50	31	trace	.69
.75	30	trace	.66
1.00	28	1.6	.77
1.75	26	1.3	.76
2.50	20	3.9	.78
3.25	10	4.7	.70
4.00	9	10.9	.81
5.00	4	12.0	.81
Medium phosphorus level (P = 60 ppm)			
0.00	32	trace	.75
1.00	29	trace	.77
2.00	24	3.3	.75
3.25	9	6.7	.84
7.00	1	12.5	.69
11.00	1	24.1	.50
High phosphorus level (P = 120 ppm)			
0.00	33	trace	.85
1.00	28	trace	.88
3.25	7	7.8	.88
7.25	3	14.7	.84

of phosphorus on the absorption of trivalent arsenic is quite different from its action on pentavalent arsenic. From a given concentration of trivalent arsenic, the tomato plants absorbed approximately the same amount of the toxic element, irrespective of the phosphorus level, and showed equal degrees of injury. In the studies with Sudan grass, the medium- and the high-phosphorus levels reduced the absorption of arsenic over that absorbed from the low level of phosphorus, with a corresponding reduction in injury. In the studies with the bean plants, the phosphorus level had some effect on the absorption of arsenic as shown in the analysis of the plant material, although it should be noted that the differences are so small as to be insignificant.

These studies reveal, therefore, that the form in which the arsenic occurs is an important factor in determining the effect of phosphorus. Hurd-Karrer (1937) in her studies on the antagonism of related ions found that sulfates more effectively reduced the absorption of selenium from selenates than from selenites. From these results she suggested that "by analogy, phosphates would be expected to have less effect on the toxicity of arsenite than on that of arsenate" (1939). The studies reported in this paper confirm that supposition.

Part III. Toxic Levels of Arsenic in Certain Hawaiian Soils

It is well known that arsenicals, when applied to soil, are far less available to plants

TABLE 5. SUDAN GRASS

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF TRIVALENT ARSENIC (AS SODIUM ARSENITE) AT DIFFERENT PHOSPHORUS LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.00	63	trace	0.44
.25	63	5.2	.36
.50	48	7.8	.59
.75	48	11.9	.45
1.00	32	18.6	.56
1.75	17	20.7	.56
2.50	5	23.1	.73
3.25	4	22.7	.48
4.00	2	42.5	.55
5.00	3	46.9	.88
Medium phosphorus level (P = 60 ppm)			
0.00	63	trace	.94
1.00	39	8.6	.58
2.00	24	10.9	.53
3.25	4	14.9	.56
7.00	3	27.5	.67
11.00	2	91.2	.81
High phosphorus level (P = 120 ppm)			
0.00	68	trace	.98
1.00	45	4.1	.88
3.25	18	12.8	.99
7.50	8	32.5	.77
15.00	2	87.5	.81

TABLE 6. BEAN

DATA FOR THE ACCUMULATION OF ARSENIC (AS PPM As_2O_3) AND PHOSPHORUS (AS PER CENT OF DRY WEIGHT), AS WELL AS DRY WEIGHTS, OF PLANTS GROWN IN SOLUTIONS CONTAINING VARIOUS INCREMENTS OF TRIVALENT ARSENIC (AS SODIUM ARSENITE) AT DIFFERENT PHOSPHORUS LEVELS.

ARSENIC IN SOLUTION	DRY WEIGHT	ARSENIC IN PLANT	PHOSPHORUS IN PLANT
ppm As_2O_3	gm.	ppm As_2O_3	% dry wt.
Low phosphorus level (P = 10 ppm)			
0.000	42	trace	0.53
.038	49	trace	.47
.075	58	trace	.44
.113	49	0.9	.44
.150	41	1.2	.50
.263	27	0.9	.69
.375	25	2.1	.63
.488	26	2.1	.56
.600	23	3.9	.63
.750	30	3.7	.50
Medium phosphorus level (P = 60 ppm)			
0.00	36	trace	.70
.3	34	trace	.70
.49	27	trace	.63
1.05	27	1.3	.63
1.65	15	4.8	.69
2.25	6	4.9	.69
2.85	5	7.0	.66
High phosphorus level (P = 120 ppm)			
0.00	31	trace	.63
.15	31	trace	.72
.49	30	trace	.59
1.13	15	trace	.66
2.25	5	3.3	.75

than they are when applied to culture solution. The degree to which arsenicals are fixed by the soil is a characteristic of the soil (Crafts and Rosenfels: 1939). The objectives of this part of the work are: first, to determine the growth reaction of certain crop plants to arsenic levels in two Hawaiian soils; second, to determine the amount of arsenic which these plants withdraw from the soil; and third, to determine whether or not it is practicable to use certain crop plants to lower the arsenic levels of those soils which have been rendered sterile to other crop plants.

Methods.—Two soils were used—one a red residual clay and the other a black alluvial soil. The red is a residual soil taken from the mountain slopes above Kailua, Oahu. It is an infertile soil which requires heavy fertilization for crop production. The black alluvial soil, taken from a papaya orchard near Kailua, is, on the other hand, extremely fertile. In fact, it was recommended to us by Dr. L. A. Dean, Soils Chemist, as being a soil whose available phosphorus level was so high that no more phosphorus could be fixed by it.[3] The growth of plants in the two soils reflected not only the difference in chemical composition, but also the difference in physical qualities, the black soil being very well adapted to pot work. These soils contained 14.7 ppm of native arsenic. This amount is added to the increments of soil arsenic shown in the accompanying tables.

The soil was dried thoroughly, screened, and ground in a plate mill. Samples of 500 grams each were weighed into No. 2 cans, after which arsenic as sodium arsenite was added in concentrations varying from 10 ppm to 3,000 ppm following the methods described by Crafts and Rosenfels (1939). The soil was allowed to dry thoroughly, after which it was removed, pulverized again, mixed, and returned to the can.

With the red soil, triplicate cans were used for each arsenic concentration for each of the three species, making a total of 225 cans. With the black soil, only one container was used for each of the two species used for each concentration of arsenic.

Tomato and Sudan grass seedlings were started on cheesecloth and transplanted to the cans when a few days old. Bean seeds were germinated in black sand and transplanted to the cans as soon as possible. With tomato and bean, two plants per can were used, and with Sudan grass five plants.

Drainage was provided by punching holes in the cans. A complete nutrient solution

[3] The phosphorus content of the two soils as extracted with 0.002N H_2SO_4 was 24 ppm for the red soil and 250 ppm for the black soil.

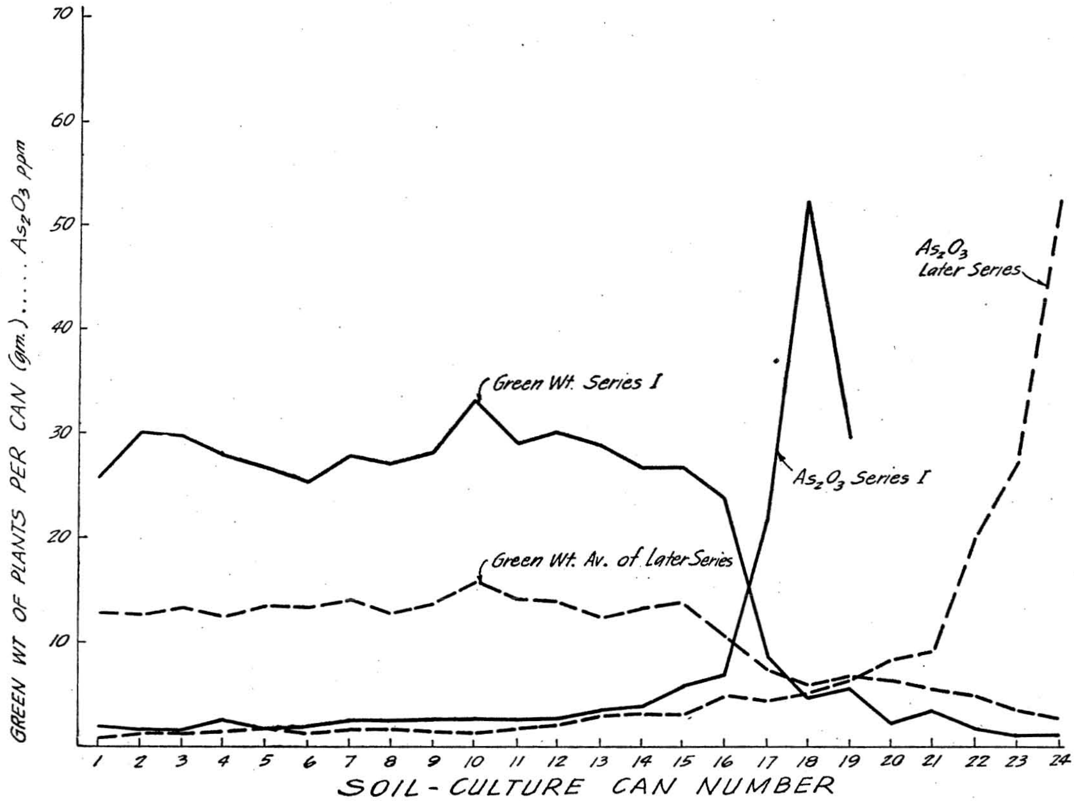

FIG. 1. Green weights and arsenic content of tomato plants grown in red soil with arsenic content ranging from 15 ppm (Can 1) to 3,014 ppm (Can 24) of As_2O_3. The difference in levels of the two green-weight curves is due to the season in which the plants were grown and is not related to treatment. (For As_2O_3 increments, see Table 7.)

was added weekly to the soil. Each crop was allowed to grow 31 days from the time of transplanting, after which the plant tops were collected and green weights obtained. The material was dried, ground in a Wiley mill, and stored for future analysis.[4]

After the removal of one crop, the soil was allowed to dry thoroughly, after which each can was emptied and the soil pulverized, mixed, and returned. Roots were in each case returned to the bottom of the can.

Results and discussion: Tomato.—The data presented in Table 7 and shown in Figure 1 for tomato plants in red soil reveal the relative tolerance of young plants to soil arsenic up to approximately 514 ppm. At higher levels the ability of the plant to grow is drastically reduced to what would be growth failure in a commercial field.

At levels below 514 ppm, growth of the tomato is approximately at uniform levels, no matter what the level of arsenic in the soil. While there may be some slight evidence of stimulation, it is very inconclusive. The arsenic content of the plant tissues varies between 2 and 3 ppm for soil levels of arsenic between 15 and 314 ppm. Between this point and approximately 414 ppm of soil arsenic, the arsenic level rises gradually

[4] All the arsenic analyses made in connection with these soil studies were made by the Chemistry Department of the Experiment Station, Hawaiian Sugar Planters' Association. The department is directed by Dr. F. E. Hance, to whom sincere thanks are due.

TABLE 7. TOMATO–RED SOIL

YIELD DATA AND ARSENIC CONTENT OF TISSUES.

CAN NUMBER	As_2O_3 CONTENT OF SOIL	SERIES I		AVERAGE OF 4 LATER SERIES	
		Green weight of plants	As_2O_3 content of tissues	Green weight of plants	As_2O_3 content of tissues
	ppm	gm. per can	ppm	gm. per can	ppm
1	15	25.7	1.8	12.8	0.9
2	25	30.0	1.6	12.5	1.3
3	34	29.7	1.6	13.1	1.1
4	45	27.7	2.4	12.3	1.3
5	54	26.7	1.6	13.4	1.5
6	65	25.3	1.8	13.2	1.1
7	74	27.7	2.4	14.0	1.5
8	85	27.0	2.4	12.8	1.7
9	95	28.0	2.6	13.7	1.5
10	104	33.0	2.6	15.7	1.3
11	115	29.0	2.6	14.0	1.8
12	214	30.0	2.6	13.9	2.1
13	314	28.7	3.4	12.2	2.9
14	414	26.7	3.7	13.1	3.0
15	514	26.7	5.8	13.7	3.0
16	614	23.6	6.9	11.3	4.9
17	714	8.7	21.7	7.3	4.1
18	814	4.4	52.3	5.9	4.9
19	915	5.3	29.6	6.6	6.1
20	1014	2.0	------	6.3	8.1
21	1514	3.1	------	5.4	9.0
22	2014	1.6	------	4.8	19.9
23	2515	0.9	------	3.2	26.9
24	3014	0.8	------	2.7	52.7

TABLE 8. TOMATO–BLACK SOIL

YIELD DATA AND ARSENIC CONTENT OF TISSUES.

CAN NUMBER	As_2O_3 CONTENT OF SOIL	SERIES I		AVERAGE OF 2 LATER SERIES	
		Green weight of plants	As_2O_3 content of tissues	Green weight of plants	As_2O_3 content of tissues
	ppm	gm. per can	ppm	gm. per can	ppm
1	15	29.0	------	------	------
2	25	30.0	1.6	38.3	0.8
3	34	32.0	1.3	39.8	1.5
4	45	27.0	1.8	41.0	1.5
5	54	29.0	2.1	39.4	1.7
6	65	26.0	2.9	40.2	2.1
7	74	32.0	5.0	38.7	1.2
8	85	28.0	2.4	41.2	2.0
9	95	29.0	2.1	37.5	1.5
10	104	30.0	2.1	39.9	1.6
11	115	29.0	5.8	34.0	1.8
12	214	30.0	2.6	33.0	2.6
13	314	31.0	3.4	39.0	2.1
14	414	28.0	4.0	38.3	2.1
15	514	29.0	5.8	34.4	3.7
16	614	31.0	5.8	35.8	5.8
17	714	28.0	6.9	31.5	9.1
18	814	24.0	8.5	34.7	4.8
19	915	25.0	8.7	38.8	11.1
20	1014	30.0	9.2	31.5	15.8
21	1514	27.0	29.6	35.9	11.9
22	2014	25.0	64.2	29.5	30.5
23	2515	22.0	103.8	28.5	35.1
24	3014	20.0	76.1	32.3	50.0

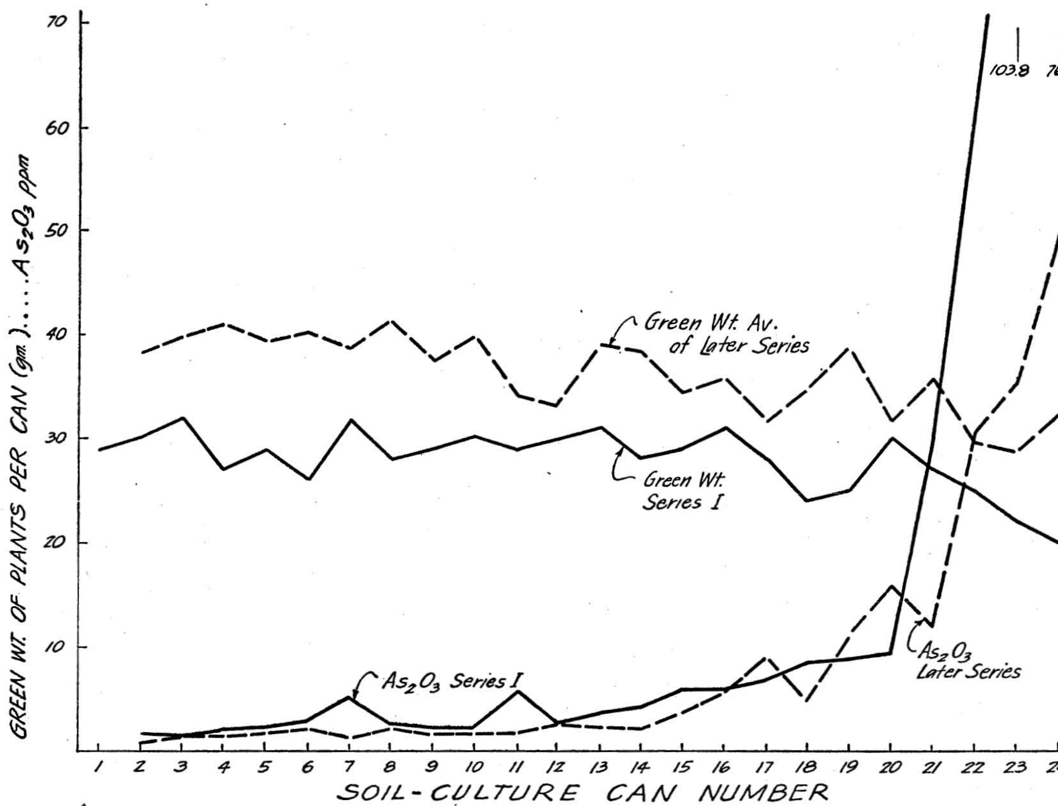

FIG. 2. Green weights and arsenic content of tomato plants grown in black soil with arsenic content ranging from 15 ppm (Can 1) to 3,014 ppm (Can 24) of As_2O_3. The difference in levels of the two green-weight curves is due to the season in which the plants were grown and is not related to treatment. (For As_2O_3 increments, see Table 8.)

from 3 ppm to 4 ppm. From higher levels of soil arsenic the plant absorbs increasing amounts of the poison, and this heavier absorption is reflected in greatly reduced growth.

Although the level of soil arsenic which might be described as critical for the tomato is the same for the later crops of plants as it is for the first crop, there is a striking difference in the amounts of arsenic absorbed by the later crops. Thus, the first crop in Can 18 contained about 52 ppm of arsenic, while the later crops in Can 18 absorbed about one tenth as much. A very much higher level of soil arsenic was necessary for the later crops to absorb 52 ppm. Two possible hypotheses suggest themselves. First, the fixation of arsenic by the soil may be a function not only of the nature of the soil but of time. Second, the soluble arsenic level of the soil may be reduced sufficiently by the first crop to reduce its absorption by later crops. However, the fact that growth in the first series as well as that in the later series is reduced in the same cultures does not lend support to the latter hypothesis.

Tomato: Black Soil.—The growth of young tomato plants in black soil, as shown in Table 8 and Figure 2, is very much better than it is in the red soil. The black soil is not only very fertile, but it possesses physical qualities which make it a better soil for pot work. The most striking contrast between the two soils is that in the black soil there is no sharp curtailment of growth even in the cans having over 3,000 ppm of soil arsenic. Although the tomatoes growing in the cans at the upper concentration levels were slightly inferior in size to those in the lower levels, they were otherwise normal in every respect. They showed no premature drying or yellowing of leaves, their roots were as extensive as the others, and yet the arsenic levels in the plant tissues were very high. In Can 24, the first crop of plants appeared perfectly normal with good color, despite the fact that the crop contained 76.1 ppm of arsenic. Like those in the red soil, though to a lesser degree, later crops of tomatoes in black soil failed to extract as much arsenic from the high arsenic soil as did the first crop.

Sudan Grass: Red Soil.—Sudan grass plants, as shown in Table 9 and Figure 3, grew uniformly well in all cans up to Can 10, in which the arsenic content of the soil was 104 ppm. There is no suggestion of stimulation in the cultures having lower concentrations of arsenic. At the higher levels of soil arsenic, Sudan grass extracted substantially higher amounts of the poison than did the tomato. The first crop of grass absorbed larger quantities of arsenic at the higher levels than did later crops. In fact, each succeeding crop of Sudan absorbed less and less arsenic from the soil, but in each crop the depression of growth occurred at the same level of soil arsenic, a fact again pointing to the idea that the actual arsenic level in the plant is not causal, though it is

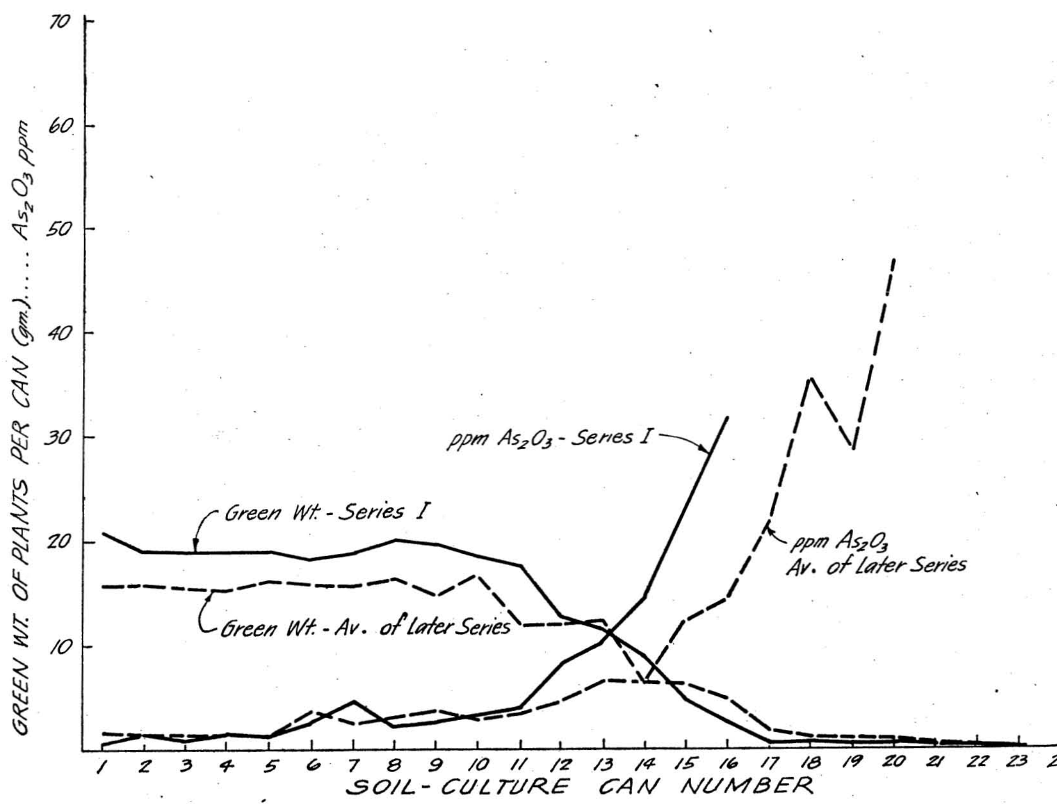

FIG. 3. Green weights and arsenic content of Sudan grass grown in red soil with arsenic content ranging from 15 ppm (Can 1) to 3,014 ppm (Can 24) of As_2O_3. The difference in levels of the two green-weight curves is due to the season in which the plants were grown and is not related to treatment. (For As_2O_3 increments, see Table 9.)

TABLE 9. SUDAN GRASS–RED SOIL

YIELD DATA AND ARSENIC CONTENT OF TISSUES.

CAN NUMBER	As_2O_3 CONTENT OF SOIL	SERIES I: Green weight of plants	SERIES I: As_2O_3 content of tissues	AVERAGE OF 2 LATER SERIES: Green weight of plants	AVERAGE OF 2 LATER SERIES: As_2O_3 content of tissues
	ppm	gm. per can	ppm	gm. per can	ppm
1	15	20.8	0.5	15.7	1.5
2	25	19.0	1.6	15.7	1.5
3	34	18.7	0.8	15.5	1.3
4	45	19.0	1.6	15.1	1.6
5	54	19.0	1.3	16.0	1.3
6	65	18.3	2.6	15.8	3.8
7	74	18.7	4.5	15.6	2.4
8	85	20.0	2.1	16.3	3.0
9	95	19.7	2.6	14.8	3.7
10	104	18.3	......	16.6	2.9
11	115	17.3	4.0	11.9	3.6
12	214	12.7	8.2	11.9	4.6
13	314	11.3	10.3	12.2	6.5
14	414	8.7	14.5	6.1	6.3
15	514	4.7	23.2	6.3	12.3
16	614	2.2	31.4	4.9	14.1
17	714	0.3	......	1.7	21.8
18	814	0.6	......	1.0	35.5
19	915	0.2	......	0.9	28.1
20	1014	0.2	......	0.8	46.1
21	1514	0.1	......	0.4	......
22	2014	......	......	0.2	......
23	2515	......	......	0.2	......
24	3014	......	......	0.1	......

TABLE 10. SUDAN GRASS–BLACK SOIL

YIELD DATA AND ARSENIC CONTENT OF TISSUES.

CAN NUMBER	As_2O_3 CONTENT OF SOIL	SERIES I: Green weight of plants	SERIES I: As_2O_3 content of tissues	AVERAGE OF 5 LATER SERIES: Green weight of plants	AVERAGE OF 5 LATER SERIES: As_2O_3 content of tissues
	ppm	gm. per can	ppm	gm. per can	ppm
1	15	25.3	2.4	36.8	1.3
2	25	22.6	5.5	33.2	1.2
3	34	23.8	5.0	31.6	1.8
4	45	25.8	4.2	32.4	1.6
5	54	23.2	3.4	33.6	1.2
6	65	24.6	3.4	32.8	2.2
7	74	27.3	......	34.5	1.3
8	85	25.8	3.7	31.1	1.6
9	95	25.2	1.3	34.1	2.1
10	104	24.6	3.4	34.9	2.1
11	115	22.7	5.8	36.3	2.4
12	214	26.4	2.1	34.3	5.4
13	314	25.6	0.4	29.6	7.7
14	414	20.5	9.2	30.0	10.7
15	514	17.7	16.1	27.4	13.5
16	614	23.7	14.0	29.0	12.9
17	714	13.5	18.5	27.1	17.6
18	814	10.5	17.4	24.6	17.4
19	915	6.8	16.1	24.4	24.6
20	1014	10.9	24.0	17.6	27.3
21	1514	4.8	28.5	10.4	29.4
22	2014	0.9	44.6	2.4	56.1
23	2515	0.1	......	1.0	86.5
24	3014	0.2	......	0.7	......

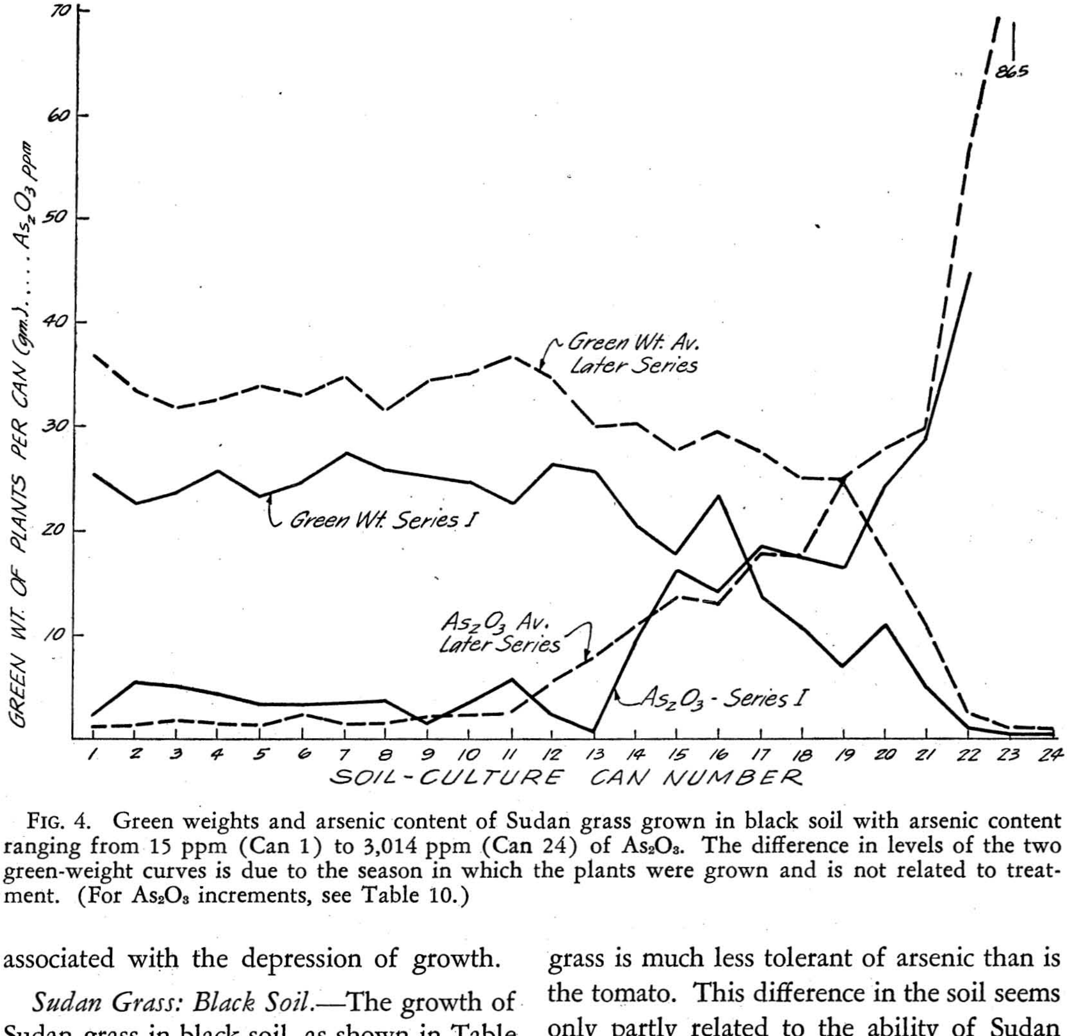

FIG. 4. Green weights and arsenic content of Sudan grass grown in black soil with arsenic content ranging from 15 ppm (Can 1) to 3,014 ppm (Can 24) of As_2O_3. The difference in levels of the two green-weight curves is due to the season in which the plants were grown and is not related to treatment. (For As_2O_3 increments, see Table 10.)

associated with the depression of growth.

Sudan Grass: Black Soil.—The growth of Sudan grass in black soil, as shown in Table 10 and Figure 4, was much more luxuriant than in the red soil. Furthermore, in the black soil normal growth occurred at much higher levels of soil arsenic, despite high tissue levels of arsenic. Unlike the curve for the tomato crops, there was a decided break in that of the Sudan grass series. Above Can 16 (614 ppm of soil arsenic) growth was progressively more difficult, and at the soil arsenic level of 2,014 ppm (Can 22) there was no growth.

Although Sudan grass in culture solution appeared as tolerant to arsenic as was the tomato, it is quite clear that in soils, Sudan grass is much less tolerant of arsenic than is the tomato. This difference in the soil seems only partly related to the ability of Sudan grass to extract higher levels of arsenic from a given soil. (Compare the arsenic levels in Figures 2 and 4 from Cans 13 to 20, in which growth of Sudan grass was still appreciable.) It is also partly related to a difference in the manner in which the plants hold the arsenic within their tissues. Thus, when the tomatoes growing in the black soil had over 20 ppm within their tissues, their growth was nearly normal. At the same tissue levels the growth of Sudan grass was nearly stopped. Thus, tolerance to soil arsenic involves root tolerance as well as tissue tolerance. Probably, root tolerance is determined in part by the nature of the root structure and in part by the intimacy as well as nature of the contact between the root surface and the soil surface bearing the arsenic. Tissue tolerance, on the other hand, may be related in part to protoplasmic structure and in part to the form in which the arsenic is held within the protoplasm after it is absorbed.

Bean: Red Soil.—The bean plant (see Table 11 and Figure 5), which in culture solution was the most susceptible of the three plants to arsenic injury, showed a tolerance to soil arsenic only slightly below that of the tomato, but considerably above that of Sudan grass.

For the bean, as for the tomato and Sudan grass, although the level of soil arsenic at which growth was sharply curtailed was the same for the first crop as for later crops, the actual amount of arsenic absorbed was greater in the first crop than in later crops. Also, the differences in the amounts of arsenic absorbed by the various crops are not all related to the differences in growth made.

GENERAL DISCUSSION

It is apparent that production of crops in heavy Hawaiian soils which have been contaminated with the herbicide sodium arsenite will be affected variously depending on the particular crop. Furthermore, crops which may be looked upon as tolerant to arsenic when grown in culture solution may become relatively susceptible to fixed arsenic in the soil. In culture solutions, the bean plant was by far the most susceptible of the three plants used, whether the arsenic was trivalent or pentavalent. The tomato was the most resistant toward pentavalent arsenic, but was about equal to Sudan grass in resistance to trivalent arsenic. In soil cultures, however, Sudan grass was considerably less resistant to sodium arsenite than either of the other

TABLE 11. BEAN–RED SOIL

YIELD DATA AND ARSENIC CONTENT OF TISSUES.

CAN NUMBER	As_2O_3 CONTENT OF SOIL	SERIES I: Green weight of plants	SERIES I: As_2O_3 content of tissues	AVERAGE OF 2 LATER SERIES: Green weight of plants	AVERAGE OF 2 LATER SERIES: As_2O_3 content of tissues
	ppm	gm. per can	ppm	gm. per can	ppm
1	15	18.7	0.5	24.3	1.5
2	25	18.0	1.1	23.7	1.5
3	34	19.0	1.8	25.9	1.8
4	45	17.3	2.4	22.8	2.2
5	54	20.7	2.9	23.5	5.0
6	65	24.0	7.9	27.5	1.8
7	74	24.7	9.0	28.0	2.0
8	85	26.7	6.9	25.2	3.2
9	95	22.7	4.8	25.9	2.1
10	104	22.3	3.4	23.0	1.7
11	115	22.3	3.7	22.8	2.1
12	214	22.0	5.5	26.7	2.5
13	314	14.0	6.3	22.7	3.7
14	414	18.0	10.3	21.2	6.9
15	514	11.0	14.0	14.2	6.6
16	614	7.7	26.4	9.5	6.9
17	714	6.3	......	11.1	10.8
18	814	9.0	24.6	6.8	12.8
19	915	6.0	35.4	6.1	12.9
20	1014	2.5	......	4.2	12.8
21	1514	2.3	......	3.2	15.7
22	2014	1.1	......	2.0	25.1
23	2515	0.7	......	1.6	32.7
24	3014	0.2	......	1.5	46.5

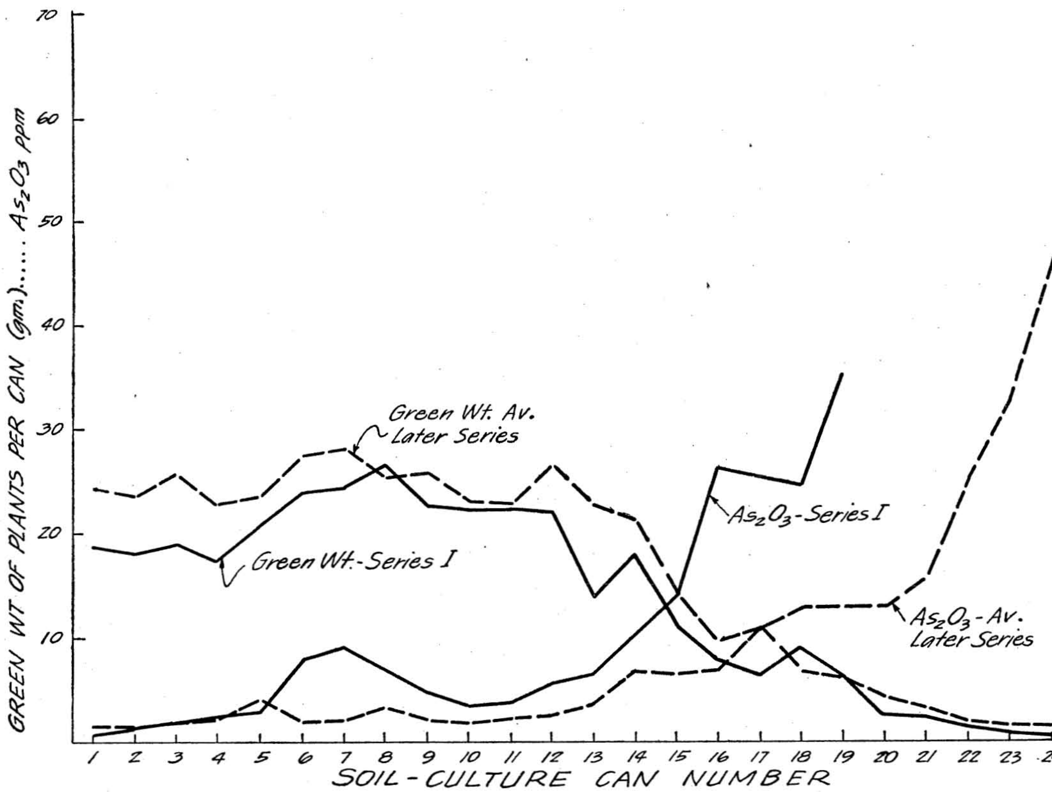

FIG. 5. Green weights and arsenic content of bean plants grown in red soil with arsenic content ranging from 15 ppm (Can 1) to 3,014 ppm (Can 24) of As_2O_3. The difference in levels of the two green-weight curves is due to the season in which the plants were grown and is not related to treatment. (For As_2O_3 increments, see Table 11.)

two. In the red soil, the arsenic concentrations which were toxic to Sudan grass, bean, and tomato were approximately 110, 250, and 550 ppm, respectively. In pounds per acre foot of dried soil, these figures become roughly 220, 500, and 1,100 pounds, respectively.

Perhaps the fact that Sudan grass is a vigorous feeder especially of fixed phosphorus in such soils is related to its greater sensitivity to soil arsenic, which probably is similarly fixed. Such an observation has support in the data presented for red soils, which demonstrate that Sudan grass extracted higher levels of arsenic from Cans 10 to 16 than did either of the other two plants. Both tomatoes and beans when grown on Hawaiian soils are fertilized heavily with phosphates in order to obtain good growth. The large grasses, however, seem to be able to take their phosphorus even though it is highly fixed.

It is doubtful whether arsenic which is applied to soils as trivalent arsenic remains trivalent after it has been in the soil for some time. Although no direct pertinent data are available from this study, indirect data may be obtained from the arsenic levels attained by the Sudan grass and tomato plants growing on the black soil. Tomato plants in black soil (Can 22) which were apparently normal contained up to 104 ppm of arsenic. Sudan grass plants in black soil in Cans 21 and 22 contained between 30 and 86 ppm of arsenic. Yet when these were grown in culture solution, only very much lower levels of trivalent arsenic were tolerated. The amounts of arsenic absorbed from the soil were more in line with the amounts of pentavalent arsenic absorbed from culture solution. It cannot be presumed, however, that after arsenic is absorbed in the pentavalent form, it remains as an inorganic compound after it becomes a part of the plant's metabolism.

The claim which has been made by many that arsenic at proper levels is stimulating to plant growth is not substantiated by the data presented here. Neither in water culture nor in soil culture is there any certain evidence of such stimulation. What slight gains from arsenic there may be are infinitesimal compared with the losses which are certain to come after the arsenic content passes critical levels.

The arsenic which was added to the soil cans was not greatly reduced in amount either by the growth of the plants or by drainage which was provided. Even the large amounts of arsenic contained in the tomato plants grown in the high arsenic cans represent very small proportions of the total amount of arsenic in the soil. To calculate the time needed to extract the arsenic from the soil through continued use of tomato plants, assuming the highest extraction observed in these tests, would require something over 100 crops. It is far better to stop the use of arsenic before critical levels are reached. It is apparent from this work, as well as from that of others, that no matter how large or how small the annual increments to the soil may be, substantially all of the arsenic remains in the tilled layer. Reducing the increment of arsenic applied merely prolongs the time of grace.

One observation needing to be brought into sharp focus is that, as shown in all figures in the text, the point of sterilization for a given crop is very much higher than the point at which crop production begins to suffer curtailment because of accumulated arsenic.

In red soil, Sudan grass began to suffer growth curtailment at about 115 ppm As_2O_3, the tomato at about 614 ppm, and the bean at about 314 ppm. From these respective points on, the increasing curtailment varies for each crop. For the tomato the further drop is precipitous, less so for the bean, and still less so for Sudan grass.

Sobering is the report from Queensland by Kerr (1939) that soil arsenic at the level of 600 ppm resulted in complete growth failure of sugar cane. In times of low prices for agricultural produce, even a 5 per cent curtailment of production due to soil arsenic may well mean the difference between profitable and unprofitable operation.

Studies carried on elsewhere have yielded some treatments which may be useful in correcting arsenic toxicity. The use of heavy phosphate applications, lime, iron oxide, and organic matter (perhaps filter cake) have shown promise of reducing the toxicity of arsenic excesses. None of these treatments reduces the arsenic content of the soil. Furthermore, most of these treatments are costly. Whether a single treatment is effective for any length of time remains to be determined.

There is only one permanent solution to the problem of arsenic accumulation so far as present-day information is concerned, and that is the cessation of arsenic applications. Substitution of other herbicides or other weed-control practices which at the moment may seem somewhat more costly may be the cheapest in the long run. Certainly there can be no reconciliation of a program of arsenic applications to the soil with any long-range view of agriculture.

SUMMARY

1. Studies made with plants treated with sodium arsenate and sodium arsenite in cul-

ture solutions show trivalent arsenic to be approximately 10 times as toxic to Sudan grass and tomato plants as the pentavalent form and approximately four times as toxic to bean plants as the pentavalent form. The trivalent form acts more quickly and violently on plant tissues.

2. Studies on the relationship of the phosphorus level to the toxicity of *pentavalent* arsenic show that an increase in the phosphorus level materially reduces the absorption of arsenic by bean, Sudan grass, and tomato plants. The phosphorus was found to have little or no effect on the toxicity of the element after it has been taken into the plant.

The phosphorus had little, if any, effect on the absorption of *trivalent* arsenic from culture solution by bean, Sudan grass, and tomato plants.

3. Results are presented for several crops of Sudan grass, tomato, and bean plants in a re-cropping experiment with red and black soils treated with increments of sodium arsenite. It was found that as time elapsed, more and more of the arsenic was fixed by the soil, a fact indicated by a reduction in the amount of arsenic found in the plant tops. Growth curtailment, however, was observed to take place each time at the same levels of soil arsenic, irrespective of the levels of arsenic absorbed.

It was found that the plant species varied in the ability to withdraw arsenic from the soil medium, tomato and bean being low and Sudan grass high in ability to withdraw the element.

4. Sudan grass and tomato plants were grown in a black alluvial soil treated with sodium arsenite, and the results were compared to those in the red soil experiment. Marked differences were found in the response of the plants to a certain concentration of arsenic in the two soils.

5. Whereas in culture solution Sudan grass was as tolerant to arsenic as the tomato and much more so than the bean, in soil, Sudan grass was less tolerant to arsenic than either.

6. The removal of soil arsenic by crops which are tolerant to arsenic will at best be a very slow process.

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